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LONG-DAYS, LIGHT-SPECTRA AND MELATONIN ALTER OVARIAN FUNCTION IN THE TELEOST FISH CHANNA PUNCTATUS (BLOCH)

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ABSTRACT

Information on light-pineal-gonadal interaction in tropical teleost species is sparse. We therefore assessed ovarian response to 1. chronic exposure to long-days, 2. red, blue and green spectra of light and 3. treatment with melatonin (given either in morning or evening) in a tropical fish during the breeding season.

Exposure to long-days (LD 14:10) increased gonadosomatic index (GSI = ovarian weight/100g body weight), number of vitellogenic follicles (VF) and decreased the number of atretic follicles (AF). Treatment with melatonin, especially when given daily in the evening prevented the long day-induced increase in the GSI ($P<0.01$). Evening melatonin decreased VF and increased AF. Exposure to red light increased ($P<0.01$). Follicular kinetics revealed an increase in the VF ($P<0.01$) and a decrease in AF ($P<0.01$) in the ovaries of fish held in the red spectrum. The fish held in green and blue light had less number of PVF ($P<0.01$) and more number of VF and AF ($P<0.01$).

Exposure to both long days and red spectrum of light stimulate ovarian function. Melatonin inhibits the ovarian activity. Observation that red light stimulates ovarian function could be of use in breeding of this commercially important species.

KEY WORDS: Photoperiod, Light spectra, Pineal Gland, Melatonin, Ovarian Follicular Kinetics.

Introduction

Melatonin chemically represents biological night (Cassone et al., 1993). In fish species the pineal, through its rhythmic production of melatonin, seems to be involved in the timing of a number of rhythmic physiological functions, especially reproduction (Joy and Agha, 1991). Electrophysiological data confirm the photoreceptor function of teleostean pineal organ (Dodt, 1963). Therefore, the pineal organ is involved in mediating the effects of photoperiod on reproduction. The photoperiodic information transuded through the pineal may lead to stimulatory or inhibitory effect on gonadal activity (Urasaki, 1972). In general long photoperiods stimulate and short photoperiod retards gonadal function (de Vlaming and Vodicnik, 1977). Since melatonin is involved in time measurement, timed administration of melatonin mimics the effect of short photoperiod (Fenwick, 1970). While the interaction of light, pineal and gonads is being recognized there are studies that show physiological effects of different colors of light in mammals (Reiter, 1991), birds (Birgersson et al., 2001), amphibians (Kovach, 1993; Joshi and Udaykumar, 1998). In fish environmental color affects growth (Levine and Mac Nichol, 1982), feeding (Dowing and Litvak, 2000), food conversion rate (Martin-Robichaud and Peterson, 1998), stress (Papoutsoglou et al., 2000) and egg development (Heichenback-Klinke, 1982), reproduction (Volpato et al., 2004). Biological effects of light and its spectra in tropical species of fish have not been studied extensively. The present study has multiple but interrelated objectives of assessing the effects of long-photoperiod, spectra of light and treatment with melatonin on the ovarian function of a commercially important tropical teleost fish Channa punctatus (Bloch).

Material and methods:

The teleost fish Channa punctatus (Bloch) were collected from Bheema river (N latitude 17) and kept for acclimatization to laboratory conditions in glass aquaria for two weeks prior to their use in the experiments. During this period they were treated with antibiotic chloromphenicol (5mg/litre of water), as prophylactic agent.

Two experiments were conducted. In both the experiments the fish were held in aquaria and placed in chambers with facility for automatic regulation of photoperiod. During the course of experiment the animals were fed with live earthworm on alternate days and the water of the aquaria changed daily. The water temperature was maintained at 21 1c. In the first experiment hundred fish were divided in to four groups of 25 each. They were held in aquaria and placed in automatically regulated photoperiod regimen. In experiment-1 a control group was placed in LD 12:12 the other three were held in LD 14:10 (long-day). Two groups of fish held in long-days (LD 14:10) received melatonin injections (daily 10 g/day) either in the morning (08.00 h) or in the evening (17.00 h). The remaining long-day group serving as control received only vehicle injection (ethanol-saline). In experiment-2 another hundred fish divided again in four groups were held in white, red (610-750nm), green (500-550nm) or blue (435-480nm) light (LD 14:10). Fluorescent lights (20W Philips) was used for white light, whereas for red, blue, or green transparent appropriately colored polyethylene paper was wrapped around the fluorescent tubes. The absorption wavelengths of the colored polyethylene papers used were determined using a constant deviation spectrometer.

The fish from both the experiments were autopsied after month-long respective treatments. The ovaries were dissected out and weighed immediately on an electronic balance and fixed in Bouin's fluid and later on embedded in paraffin wax for histological study. Five µ paraffin sections were stained in Haematoxylin and eosin. The Gonadosomatic index (GSI) was calculated as; gonadal weight / body weight x 100.

Follicular kinetics was studied in histological sections of the ovaries. The follicles were classified as previtellogenic follicles (PVF), vitellogenic follicles (VF) or atretic follicles (AF) based on their histomorphology. At least eight fish from each group were used for this purpose. The counting of the follicle was done in 20 slides from anterior, middle and posterior segments of each ovary.

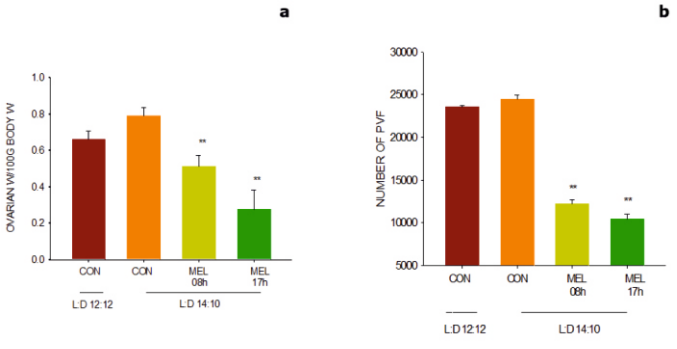
The statistical analysis was done using a computer program for ANOVA and Schiff's Pair wise comparison test.

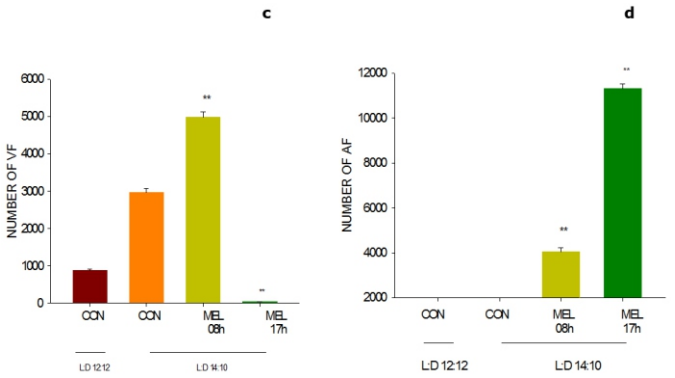
Results:

In experiment-1 the GSI (Gonadosomatic index) increased ($P<0.01$) in the fish that were held in long-days LD 14:10 (Fig. 1a) when compared to the GSI of fish held in LD 12:12. Treatment with melatonin (10µg) prevented the long-day-induced increase in GSI ($P<0.01$) in both morning and evening injected groups, however the decrease was more distinct in the fish that were treated daily in the evening.

Follicular kinetics study clearly indicates an increase ($P<0.01$) in the number of VF (Fig.1c) and decrease ($P<0.01$) in the AF number (Fig. 1d) in the ovaries of the fish held in long-days, however the number of PVF number remained unaltered (Fig. 1b).Fig. 1.

Fig. 1.



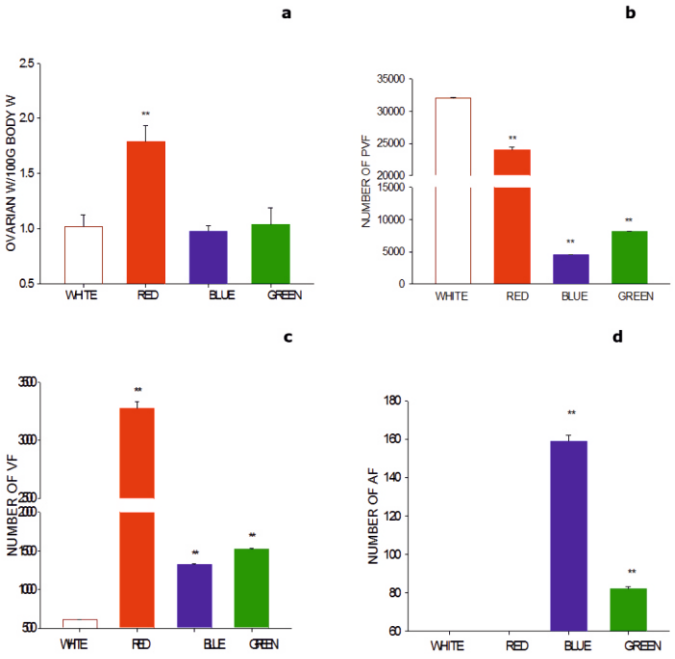


Treatment with melatonin in the evening resulted in a significant decline (P<0.01) in the number of PVF and VF and maximum increase (P<0.01) in the number of AF. The fish that received melatonin treatment in the morning had a decrease (P<0.01) in the PVF and increase in VF and AF number.

In experiment-2 exposure of fish to red spectrum of light stimulated the GSI (P<0.01), other spectrum of light (blue or green) had no significant effect on the GSI (Fig. 2a).

Data on follicular kinetics study reveals that exposure of fish to red, blue or green spectra of light lead to a decrease (P<0.01) in the number of PVF when compared to control fish held in white light (Fig. 2b). Though the number of VF increased (P<0.01) in fish that were exposed to red, blue or green light (Fig. 2c), the increase was more striking in red light exposed fish, in addition number of AF was almost nil in control as well as red light exposed fish. In fish held in blue and green spectrum the AF number increased (P<0.01) (Fig. 2d).

Fig. 2.



Discussion:

The result of experiment-1 suggests that long-days (LD 14:10) stimulate ovarian growth in *Channa punctatus* (Bloch). In *Heteropneustes fossilis* also long days stimulated whereas short-days or treatment with melatonin inhibited ovarian growth (Sundararaj and Keshavanath, 1976). Ghosh and Nath (2005) reported reduced GSI following melatonin treatment in the catfish *Clarias batrachus*. An acceleration of gonadal growth was observed also in *M. tengara* (Guraya *et al.,* 1976) and even in *C. punctatus* (Srivastava and Singh, 1992) following exposure to long photoperiods and continuous light (LL).

In the present study long-days stimulated and timed injections of melatonin inhibited gonadal function. Melatonin seemed to be more effective in inhibiting gonads when administered daily late in the afternoon. Daily morning injections did inhibit gonads but inhibitory effect was not as marked as seen in the evening treated group. Injections of melatonin daily in the evening are known to augment the duration of nocturnal endogenous peak of melatonin (Reiter *et al.,* 1986). Nocturnal duration of melatonin is believed to represent biological night at least in mammals (Arendt, 2006). Therefore increased melatonin may signal for increased dark phase of the day and night cycle; in other words it may mimic short photoperiod. It is well known that in long-day breeders short photoperiods suppress gonads. The situation could be the same in non-mammalian vertebrates. The reduced gonad-suppressing ability of morning-administered melatonin could be due to down-regulation of melatonin receptors in the morning hours. Further experimental studies will be needed to verify this hypothesis.

In experiment-2 the GSI increased significantly in the fish that were held in red light while other colors of light (blue or green) did not affect the GSI significantly. But a study on *Oreochromis niloticus* (Volpato, 2000) reported that the proportion of reproducing fish was significantly higher (6 of 13) in the group exposed to the blue color compared to the group exposed to white color (1 of 12). It was concluded that reproduction in the presence of blue light was more frequent and intense than in the presence of white light; however the experiment was conducted using only blue color what would have happened in other colors is a moot point. Another study (Volpato and Barreto, 2001) on *Brycon cephalus* reported that a significantly larger number of females spawned when hormonally induced reproduction occurred in a green environment compared to a white environment (8 of 9 Vs 4 of 9). In the present study however assessed the effects of light spectra on follicular kinetics and not spawning activity. It reveals that red light increased percent ovarian weight (GSI) while blue or green color did not. Earlier study from this lab (Joshi and Udaykumar, 1998; 2000) reported that red light stimulates GSI in frog *Rana cyanophlyctis* where in the Indian gerbil *Meriones hurrianae* (Sinhasane and Joshi, 1998) Green light stimulated the gonads. It is evident from all these studies that wavelengths do influence reproduction but the responses may vary with the species and its habitat.

The data from follicular kinetics study reveals that red light exposed fish had higher number of VF and very few atretic follicles. Since increase in the numbers of VF and a concomitant decrease in AF indicates stimulation of ovarian function, the observation further strengthens our conclusion that red wavelength stimulates gonadal function in this fish. Exposure of the fish to blue and red spectra also lead to an increase in VF when compare to the ovaries of fish held in white light but there was an associated increase in AF suggesting enhanced follicular atresia.

However, the mechanism(s) whereby red light influences gonadal activity remains unclear. Daily exposure to red light probably affects melatonin turnover or the rhythmic pattern of its production and this in turn may modulate reproductive function. It is believed that in vertebrates the pineal gland through its rhythmic production of melatonin mediates biological effects of light. The duration of nocturnal melatonin is involved in the measurement scotophase of the diurnal cycle. If the physiological effects of different spectra of light are mediated through alteration in melatonin rhythms remains to be understood.

The observation that red light stimulates ovarian function could be of use in breeding of this commercially important species.

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